

Recombinant Protein Expression

Materials and Equipment

- Microtube rack
- 15 ml conical tubes
- Micropipette
- Micropipette tips
- Microtubes (0.6 ml)
- Laminar flow hood
- Shaker
- Centrifuge

Reagents

- 50 ml liquid LB medium with proper antibiotic (KAN 15 mg/ml, CAM 35 mg/ml, AMP 100 mg/ml)
- IPTG 1 M

Methodology

1. 2 different and separated CFU obtained through a Stria culture (corresponding to a cassette assembly that will be induced and analyzed according to its solubility) were inoculated into 15 ml test tubes with LB broth (liquid) + proper antibiotic. Incubate them at 37°C overnight.
2. Inoculate 4 ml of the previously mentioned culture into a 50 ml flask with LB broth (liquid) + antibiotic. Incubate 1 hour at 37°C, 200 rpm.
3. Perform lectures at a 600 nm wavelength via a spectrophotometer until reaching a 0.8-1.0 absorbance value.
4. Take 1 ml sample and place on ice.
5. Add 50 µL of 1M IPTG to the 50 ml flask.
6. Return to incubating conditions and take a sample each hour for 4 hours.
7. Centrifuge all samples and recover biomass (even the first sample that was not induced with IPTG) at 13,000 rpm for 5 min.
8. Resuspend the biomass in 40 µL of Laemmli buffer, then put the samples into boiling water for 5 minutes.
9. Cool the samples at room temperature and then put them on ice (or store them at -20°C)
10. SDS PAGE is carried out.